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PB-283 050

Fish Cough Response - A Method for Evaluating Quality of Treated Complex Effluents

Environmental Research Lab -Duluth, Minn

18 Jul 77

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3.
EPA-600/J-78-015	JOURNAL ARTICLE	PB 283 050

4. TITLE AND SUBTITLE
Fish Cough Response -- A Method for Evaluating Quality of Treated Complex Effluents

5. REPORT DATE

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
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8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
U.S. Environmental Protection Agency
Environmental Research Laboratory-Duluth
6201 Congdon Boulevard
Duluth, Minnesota 55804

10. PROGRAM ELEMENT NO.
1BA608

11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS
Same

13. TYPE OF REPORT AND PERIOD COVERED

14. SPONSORING AGENCY CODE
EPA-600/03

15. SUPPLEMENTARY NOTES Reference: Carlson, Richard W., and Robert A. Drummond. 1978. Fish Cough Response -- A Method for Evaluating Quality of Treated Complex Effluents. Water Research Vol. 12, pp. 1-6.

16. ABSTRACT

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NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Freshwater fishes Cough Respiration Ventilation Toxicity Effluents Methodology Monitoring	Electrode chambers Opercular movements Muscle action potentials Test procedures	06/C 06/F 06/T 14B 14C

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	8
	20. SECURITY CLASS *(This page)*	22. PRICE PCA02 MFA01
	UNCLASSIFIED	

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

Water Research Vol. 12, pp. 1 to 6. Pergamon Press 1978. Printed in Great Britain.

EPA-600/J-78-015
JOURNAL ARTICLE

FISH COUGH RESPONSE—A METHOD FOR EVALUATING QUALITY OF TREATED COMPLEX EFFLUENTS

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(*Received* 18 *July* 1977)

Abstract—Bluegill sunfish (*Lepomis macrochirus*) showed increases in cough frequency commensurate with effluent concentration when exposed for 24 h to different industrial and municipal effluents. Effluents known to be toxic caused steadily increasing cough rates in the fish as effluent concentration approached acutely toxic levels. Bluegills exposed to three different samples of effluent, collected from the same source over an 8-month period, showed decreased levels of cough rate as the effluent improved in quality. Cough frequency is a rapid and sensitive physiological characteristic for evaluating the quality of industrial and municipal effluents and variation in quality of same-source effluents before discharge into receiving waters.

INTRODUCTION

Dischargers and regulatory agencies need a rapid biological test method for evaluating the quality of treated industrial and municipal effluents before they are discharged into receiving waters. Evaluation of effluent quality by physical–chemical measurements does not provide information regarding the possible synergistic or antagonistic effects that complex effluents may have on aquatic life. The acute fish bioassay, designed to establish lethality in terms of 96 h LC_{50} values, is a commonly used biological indicator of effluent toxicity. However, the test is insensitive to those levels of effluents that may cause deleterious effects if exposures are continued for prolonged periods. What is required, then, is a test method that is not only rapidly sensitive to acutely toxic effluent levels, but will also provide information about sublethal concentrations within the time frame of an acute bioassay. Cough frequency of fish is a sensitive, easily measurable physiological characteristic that may fulfill these requirements.

Sprague (1971) noted in his review that respiratory impairment in fish is a good indicator of toxicant stress, but that rate of coughing shows more promise as a significant response to sublethal concentrations than opercular rate. Fish respond to several metals (Dandy, 1967; Martens *et al.*, 1970; Skidmore, 1970; McPherson, 1973; Hughes, 1975; Sellers *et al.*, 1975) and organic compounds (Schaumburg *et al.*, 1967; Bull & McInerney, 1974; Lunn *et al.*, 1976) with an increase in cough frequency. The cough response is also a short-term predictor of long-term adverse effects on fish exposed to copper (Drummond *et al.*, 1973) and mercury (Drummond *et al.*, 1974). Little work has been done in which the cough response has been used to assess effects of industrial effluents on aquatic life. However, Schaumburg *et al.* (1967); Walden *et al.* (1970) and Davis (1973) studied pulp-mill effluents and found that increases in cough frequency were proportional to effluent concentration.

The main objective of this study was to measure the cough response of bluegill sunfish (*Lepomis macrochirus*) exposed to a variety of treated complex industrial and municipal effluents, and to determine whether or not the response is an applicable bioassay tool for evaluating effluent quality. To accomplish this goal, existing methods and procedures were modified to measure the respiratory functions of fish exposed to a complex effluent. To date, nearly all cough data available has been produced by measuring the buccal water pressure of cannulated fish exposed to single toxicants, or in a few cases, to complex effluents (Saunders, 1961; Schaumberg *et al.*, 1967). Although this method is reliable, it is too cumbersome to use as a routine monitoring device (Walden, 1976). Spoor, Neiheisel & Drummond (1971) developed an alternative to indwelling cannulae for measuring the respiratory responses of fish. Their electrode chamber uses stainless steel, unimplanted wire electrodes to detect the muscle action potentials associated with the respiratory movements of a free-swimming fish. But, while this method has been successful when used with fish exposed to single toxicants, it has never been used to study the respiratory responses of fish exposed to a complex effluent. Therefore, a test of the electrode chamber method as a monitoring device for complex effluents was an inherent part of the primary objective.

A second objective was to measure the cough response of bluegills exposed to effluent samples collected from the same source, but at different times of the year. This would yield some information on whether or not the response could be used to detect variations in quality of a single complex effluent over time.

MATERIALS AND METHODS

Test apparatus

The experimental apparatus for studying the cough response of fish to treated effluents included an electrode chamber designed after that described by Spoor *et al.* (1971). The chambers were constructed of single-strength glass, silicon glue, and stainless steel wire electrodes. Shielded two-conductor cable connected each electrode chamber to an automatic switch and then to high-gain preamplifiers. Fish movements, including opercular and the cough reflex, were recorded on rectilinear strip-chart recorders. The cough reflex occurred periodically as a spike associated with two to four rapid opercular movements in the normal rhythmic ventilating pattern and was most easily differentiated when recorder paper speed was set at 0.1 cm sec^{-1}.

Each electrode chamber (15 cm long × 4 cm wide × 10.5 cm high) exposed a fish to effluent or control water airlifted up glass tubing from an effluent tray into the chamber (Fig. 1). One electrode was glued to the drain end and the other electrode mounted on a moveable glass baffle. Suspending the baffle from one of three sets of notches permitted interior chamber size to be adjusted to accommodate fish 3–12 cm long. Glass lids covered each chamber except for the water inlet, and a sliding glass standpipe adjusted chamber volume to 250 ml.

Four electrode chambers were placed in a three-sided plywood box constructed so that the drain end of each chamber extended beyond the bottom on the open side. The box rested on the sides of a water bath containing an effluent tray that was divided into two equal compartments. Volume of each compartment was 2000 ± 50 ml when filled to a drain hole, and each compartment supplied water or effluent to two electrode chambers.

Air was supplied through glass tubing bent 180° at one end to fit into the bottom of the lift tube. Air flow was regulated with three-way valves, and when properly adjusted a maximum of 65 ml min^{-1} was possible. Flows were maintained at approximately 50 ml min^{-1} through each chamber during testing, and each chamber drained directly back into the effluent tray.

The test apparatus used for monitoring bluegill cough response to effluent samples collected from a single source, but over time, differed from the above in that the electrode chambers were constructed in units of four instead of individually. These four-compartment chambers had stainless steel screen electrodes covering the open ends of each compartment, and were designed to be immersed in each of five, 14 l., aerated static test concentrations and a control. This preliminary test design was abandoned for the airlift system which required less effluent per experimental run, but, because the first sample of the series was tested with this design, the apparatus was retained so that the second and third samples collected over an 8 month period would be tested under the same conditions.

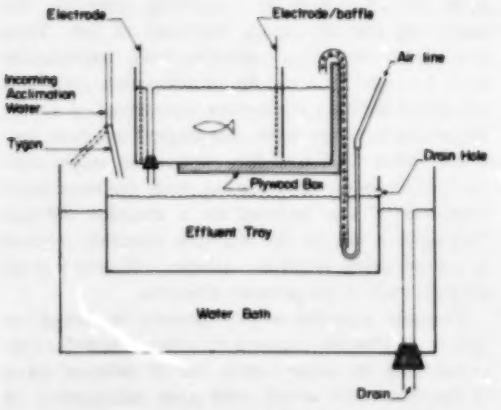

Fig. 1. Schematic diagram of the individual electrode chamber test apparatus for measuring the cough response of fish to treated complex effluents.

Biological methods

Lake Superior water used for acclimation of test fish had a pH of 7.5 ± 0.2 and a total hardness of 44–46 mg l^{-1} (as $CaCO_3$). The water was charcoal filtered before it was aerated in a series of reservoirs to maintain dissolved oxygen close to air-saturation. Temperature was adjusted to 20.0 ± 1.0°C, and the water was delivered to each effluent-tray compartment at 85 ± 10 ml min^{-1} for airlift delivery to electrode chambers.

Bluegill sunfish (*Lepomis macrochirus*) were obtained from stock hatched and raised at the Environmental Research Laboratory, Duluth. The average length was 94.5 mm and average weight, 11.2 g. The fish were maintained in holding tanks at proper temperature and photoperiod for several weeks before they were used and were fed frozen brine shrimp along with a pelletized dry food.

Light was supplied by a combination of ceiling fluorescent and incandescent bulbs directly over test chambers. Blugill diel respiratory patterns (Sparks *et al.*, 1972a) were almost eliminated with a constant 24 h photoperiod.

Test procedures

Secondary or equivalent treated effluent samples were shipped to the laboratory via air freight in 32-liter stainless steel containers. Receiving stream water was collected above the point of effluent discharge and shipped in a separate container. Any effect on cough response due to receiving water could be assessed by direct comparison to Lake Superior water controls. Effluent and receiving-water samples were brought to test temperature before the test was started. The pH of effluent and receiving water samples ranged from 6.6 to 7.5 before dilution, except for the mine wastes which were both slightly above pH 8.0. Dissolved oxygen levels were maintained above 6.0 mg l^{-1} during testing.

Fish were allowed to acclimate in electrode chambers for 3 days under flow-through conditions in Lake Superior water, which was long enough for their cough rates to decline to a normal level of approximately one per minute. The water-delivery tubes were switched from effluent trays to surrounding water baths so that incoming water maintained test temperature. Two fish were used per effluent concentration and controls for a total of 16 fish per experiment. Test concentrations were selected from a logarithmic series and expressed in accordance with standard methods (American Public Health Association, 1971) Acclimated test fish were allowed to remain in Lake Superior water to eliminate stress due to transfer while acclimation water was siphoned out of each effluent-tray compartment and replaced with a mixture of effluent and receiving water to provide the following proportions:

Per cent effluent	Per cent receiving stream water	Per cent Lake Superior water
75	0	25
56	19	25
32	43	25
18	57	25
5.6	69.4	25
1.0	74	25
0	75	25
0	0	100

Table 1. Percentage change in cough rate of bluegill sunfish (*Lepomis macrochirus*) after 24 h in various treated effluents and control waters

Effluent concentration (%)	Pulp mills			Carpet mill	Chlorinated municipal	Chemical plants			Coal mine	Uranium mine
	A	B	C			A	B	C		
75	251	456	581	323	117	63	†	*	†	*
56	358	404	347	213	126	56	†	*	†	*
32	185	440	287	108	128	46	166	†	67	*
18	118	370	189	67	107	26	88	206	46	*
5.6	73	69	114	104	131	16	36	386	36	†
1.0	28	21	26	75	72	6	46	5	62	505
Receiving stream water	-3	31	17	53	133	11	16	30	37	14
Lake Superior water	0	9	10	15	17	-12	10	-16	12	3

*Fish died during test

†Loss of signal

Test procedures differed slightly for monitoring variation of a single effluent. Because the first sample was lethal at 12.0% effluent, concentrations of 12.0, 6.0, 3.0, 1.5 and 0.8% effluent plus a control were selected for cough tests. Four fish were used per concentration and control, and were acclimated under flow-through conditions in 14 l. test tanks. Incoming water to a spare tank was stopped, water was siphoned from the tank and replaced with effluent to the correct concentration, and the four-compartment electrode chamber along with the acclimated fish were transferred into the test tank. This procedure was repeated until all concentrations and a control were set up to begin the test. Each static tank was slowly aerated to promote circulation through the electrode chamber.

All experimental and control fish were monitored during six regularly scheduled hourly periods beginning at the end of the acclimation period. Each polygraph monitored four fish simultaneously for 15 min per scheduled period before automatically switching to the next group of fish. A representative 5 min section of the strip-chart recording was selected and coughs per minute counted for each fish. A mean cough frequency for each group of two or four fish was computed for the day before testing (day 0) and for each successive 24 h period of exposure (day 1, day 2). The percentage change in cough frequency from the mean cough frequency on day 0 was calculated for each day of the 2 day exposure and was used to compare the rate of coughing for each group of fish to its pre-exposure level.

RESULTS

The cough frequency of blugill sunfish increased when they were exposed to each of 10 secondary or equivalent treated complex effluents at concentrations ranging from 1.0–75% (Table 1). However, the level of response to the effluents tested differed considerably. Chemical plant *A* elicited the least response; cough frequency increased only 6–63% over the entire concentration range during the first 24 h of exposure. In contrast, bluegills died in all concentrations at and above 18% uranium mine effluents and 56% chemical plant *C* effluents. Also, increases in cough frequency were large at the lower concentrations of these effluents. A 505% increase in cough rate occurred at 1.0% uranium mine effluent, and coughing increased 386% at 5.6% chemical plant *C* effluent. Comparable increases in cough rate occurred only in the higher concentrations of effluent samples collected from the carpet and pulp mills.

Table 2. Percentage change in cough rate of bluegill sunfish (*Lepomis macrochirus*) after 24 h in an ammunition plant wastewater and control waters

Effluent concentration (%)	Change in cough rate (%)
1.0	1,131*
0.75	1,225*
0.56	774
0.32	258
0.18	182
0.06	143
Receiving stream water	91
Lake Superior water	49

*Fish died during test

Preliminary tests showed a pre-charcoal-treated ammunition plant wastewater to be toxic, so concentrations were lowered to appropriate levels for cough-response testing (Table 2). One of two fish died in each of the two highest concentrations, and this effluent also caused the largest percentage increase in cough frequency from pre-exposure levels, reaching 1,225% at the 0.75% concentration.

Simultaneous on-site bioassays conducted by regional Environmental Protection Agency personnel reflected the extremes in cough response to the chemical plant *A* and ammunition plant effluents. In a 96 h flow-through bioassay 29 out of 30 bluegills survived in 100% chemical plant *A* effluent. The 96 h LC_{50} value for fathead minnows (*Pimephales promelas*) exposed to the ammunition plant wastewater was 1.1% effluent in an aerated static test.

Although bluegill cough frequency usually increased with increasing effluent concentration for each sample, some variation in response was noted. For example, cough rate declined as concentration rose from 56 to 75% pulp mill *A* effluent. Most of these deviations were attributed to individual tolerance in fish and the small number of fish tested at each concentration. The chlorinated municipal effluent also caused increases in cough frequency, but at approximately the same level for all concentrations. In this case bluegill cough response was affected to about the same extent by both the effluent sample and its corresponding receiving water at 75% concentrations. Because this was the only sample in which receiving water exerted a considerable effect, cough response due to receiving water only was not prorated out for each concentration of the different effluents tested.

The cough-frequency data presented in Tables 1 and 2 are only for day 1. Cough rates declined on day 2 for all samples except pulp mill *A*, carpet mill, and chemical plant *C*. Cough frequency remained at approximately the same level as day 1 for these effluents. Decline in cough rate was probably due to decreasing toxicity of the effluent samples over time.

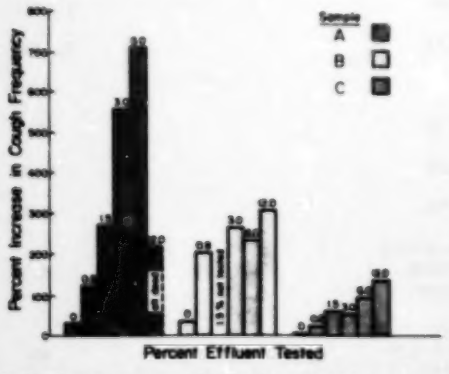

Fig. 2. Cough response of bluegill sunfish (*Lepomis macrochirus*) exposed to three different effluent samples collected from a class D oil refinery over an 8-month period.

Loss of volatiles and easily oxidized compounds was to be expected with the airlift system. The system also had a high loading factor (8.8 g l^{-1}) so the fish may have been detoxifying some of the effluent samples.

Four effluent samples caused a considerable loss in signal amplitude at higher concentrations, and any additional increases in cough frequency could not be determined. The effluents may have physically fouled the electrodes, or loss of signal was related to effluent conductivity. In addition, the fish may have been severely stressed and thus were emitting extremely low ventilatory signals.

Bluegills exposed to three effluent samples collected from a class *D* oil refinery at 4-month intervals showed considerable variation in cough response due to the different samples (Fig. 2). Sample A was lethal at 12.0% effluent and increases in cough levels of survivors ranged from 122–709% over the concentration range 0.8–6.0% effluent. Samples *B* and *C* were not lethal at 12% effluent, and changes in cough rate were greatly reduced from those shown in sample *A*. Percentage increase in cough rate reached only 311% at 12.0% effluent for sample *B*, and sample *C* elicited increases of only 28–144% at 0.8–12.0% effluent.

DISCUSSION

We believe a large and rapid increase in cough rate of fish exposed to a treated complex effluent indicates potential toxic effects. Research with single toxicants has shown this correlation. Hughes (1975) found that rainbow trout (*Salmo gairdneri*) exposed to 20 ppm zinc died in 3–4 h, but not before their cough frequency increased from about 2 m^{-1} to above 25 coughs m^{-1}, or 1,150%. Sparks *et al.* (1972b) demonstrated a graduated response in cough rates of bluegill sunfish exposed to a concentration range of 3–40 mg l^{-1} zinc. In that test increase in cough rate exceeded 200% throughout a 24 h exposure at 6 mg l^{-1} zinc, a concentration just above the LC_{50} value determined by Pickering & Henderson (1966). Copper affected brook trout (*Salvelinus fontinalis*) similarly. At a concentration of 115 mg l^{-1} Cu^{2+} (slightly above the 96 h LC_{50} reported by McKim & Benoit in 1971) coughing increased approximately 270% after a 20 h exposure (Drummond *et al.*, 1973). In all cases the effects were apparent within a few hours after the start of exposure.

In this study large and rapid increases in cough rate occurred at 1.0% uranium mine effluent and in the death-causing concentrations of the ammunition plant wastewater. Cough frequency did not reach as high a level in chemical plant *C* effluent, but still exceeded 300% at only 5.6% effluent. Large and rapid increases in cough rate also occurred in effluents that did not cause death. Levels of coughing were high in fish exposed to all of the pulp mill effluents in higher concentrations, as well as in those exposed to 75% carpet mill effluent. These observations indicated that the effluents were approaching toxic conditions

and agree well with those of Walden *et al.* (1970), who found that cough rates of rainbow trout exposed to bleach-kraft pulp-mill effluent rose steadily as concentration approached the 96 h LC_{50} value.

Lower levels of change in cough frequency may be more prognostic of sublethal effects. McKim & Benoit (1971) determined the "safe" concentration of copper to be 9.5 $\mu g\ l^{-1}$ for brook trout. Significant increases in cough rate of brook trout occurred only above 9.0 $\mu g\ l^{-1}$ copper in a 20 h exposure (Drummond *et al.*, 1973). Davis (1973) exposed sockeye salmon (*Oncorhynchus nerka*) to bleach-kraft pulp-mill effluent and found that increases in cough rate began at 20% of the 96 h LC_{50}; this was the same concentration at which other workers reported reduced specific growth rates for this species. However, chronic bioassay data were not available for the effluents tested in this series, and correlations could not be established between cough response and sublethal effects. Fish cough rates increased at all concentrations of all effluents tested, but we did not determine the percentage increase in cough rate that marked the breakpoint between a "no-effect" level and a concentration that may have long-term adverse effects if exposure were continued for a prolonged period of time.

Although the ecological significance of increased cough frequency in fish due to sublethal effluent exposure has yet to be determined, some judgments can be made concerning effluent quality. The pattern of bluegill cough response suggested that most complex industrial and municipal effluents could be categorized qualitatively. Three major classifications of effluents were apparent in this study. Effluents of poor quality included those of known toxicity that caused high cough levels in survivors at low concentrations (ammunition plant, chemical plant *C*, and uranium mine). All the pulp mill effluents and the carpet mill and chemical plant *B* effluents could be placed in a group of intermediate quality effluents. Fish exposed to these effluents showed steadily increasing, high cough levels as concentrations increased that imply sublethal, if not lethal, effects should exposure be prolonged. The remaining classification includes those effluents that caused little or no increase in cough frequency, even at higher concentrations, such as the coal mine, chemical plant *A*, and chlorinated municipal effluents. Although samples of these effluents appeared to have little effect on bluegills relative to the other effluents tested, small increases in cough rate may yet indicate long-term sublethal effects.

Fish cough response is also a useful endpoint for determining whether or not an effluent is of consistent quality over time. The resulting increases in cough frequency (Fig. 2) of bluegills exposed to three effluent samples collected from a class *D* oil refinery agreed well with observations made by treatment-plant operators concerning the treated effluent. Sample *A* was collected shortly after an explosion at the refinery had seriously disrupted the normal effluent treatment process. The sample was lethal at 12% effluent, and high cough levels at remaining concentrations showed it to be of poor quality. The treatment facilities had returned to normal operation after 8 months, and sample *C* was considered to be most representative of their final treated effluent. Although bluegill cough rates remained high in sample *C*, they were considerably less than those of samples *A* and *B*. The results substantiate the plant operators' opinions that effluent quality was improving.

We conclude that fish cough response is a good physiological endpoint for assessing the quality of treated complex effluents because, first of all, the methods and procedures for measuring cough rates are readily amenable as a routine laboratory monitoring tool. Electrode chambers are simple and straightforward to construct, the instrumentation required to record respiration data is easy to operate, and there are no elaborate surgical procedures to perform, such as is necessary when using cannulated fish to obtain the cough data. Secondly, there is the speed of the response. This is especially important to regulatory agencies which must assess effluents from a tremendous number of industries to ensure that they are fulfilling their National Pollutant Discharge Elimination System permit requirements. Effluent samples from an array of industries can be tested at a central location and those judged to be of poor quality on the basis of impaired respiration can be quickly singled out for further testing to establish toxicity. Even with an acclimation time of three days, the test does not exceed the 96 h required to complete an acute bioassay. Third, fish can act as a sensor for those industries that are discharging good quality effluents. A dramatic increase in cough rate over a short time period can serve as a warning that potentially toxic conditions are developing in the effluent. Finally, there is no sublethal test that, as yet, satisfies the demand for one that is "ecologically relevant". However, a tentative relationship between adverse long-term effects due to chronic exposure and significantly increased cough rates in fish has been established for copper (Drummond *et al.*, 1973) and mercury (Drummond *et al.*, 1974). Also, Walden (1976) has shown that impaired respiration in fish is a major effect of kraft mill effluents, and he contends that the respiration bioassay is undoubtedly ecologically relevant. While coughing in fish may not be meaningful for all types of pollutants, we believe that future research will validate the ecological significance of the cough response for many toxic materials, and that mode of action studies will further define the apparent relationship between toxicity and the cough reflex.

Acknowledgements—We are grateful to Leon Meyers and Fred Pfeffer for their technical support. The efforts of EPA regional personnel who provided us with effluent samples are appreciated. We also extend thanks to Barbara Halligan for drafting figures and Thomas Jackson for maintaining the test apparatus.

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